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NOTES AND COMMENTS

Israeli acute paralysis virus in Africanized honey bees in southeastern Brazilian Apiaries

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Honey bee losses in Brazil have been observed over the past few years. These losses share somewhat similar symptoms with the syndrome known as Colony Collapse Disorder (CCD) in the USA. After more than a half century of introgression from *Apis mellifera* subsp. *scutellata*, Africanized honey bees have tolerance against *Varroa destructor* and other parasites and other pathogens (Aumeier *et al.*, 2001; Guzmán-Novoa *et al.*, 1999; Mondragón *et al.*, 2005; Moretto and Mello, 1999; 2001; Rosenkranz, 1999; Vandame *et al.*, 2002). Consequently, it was surprising when serious population declines were observed in southern Brazil. Specifically, decreased bee populations and significant colony losses have been observed in São Paulo state, generally between March and July (autumn and winter in the Southern Hemisphere). We are actively testing these bees for indicators or causes of these losses, and for any similarities between colony losses in Brazil and CCD as described in the USA (vanEngelsdorp *et al.*, 2009). This disorder, whereby the majority of worker bees in a colony disappear over a matter of weeks, often leaving healthy brood behind, has been tied to losses of 30-90% of colonies from some beekeeping operations in the USA (vanEngelsdorp and Meixner, 2010). Cox-Foster *et al.* (2007) used a metagenomic approach to survey microbes in CCD hives, normal hives, and imported royal jelly. Candidate pathogens were screened for significance of association with CCD, and IAPV, a positive stranded RNA virus belonging to the genus *Aparavirus*, family Dicistroviridae, displayed a strong correlation with CCD in surveyed colonies.

The role of IAPV in honey bee losses remains controversial as IAPV has been found in pre-CCD historical samples from the USA (Chen and Evans, 2007), and other surveys have not established any association with this virus. There is general agreement amongst bee-keepers and scientists that no single factor is responsible for the dramatic losses of honey bees in general or CCD specifically (Neumann and Carreck, 2010). Whilst it is believed that colony losses arise from a combination of multiple factors, there is, however, strong evidence for a role of viruses in this malady. Many honey bee viruses have been found in Brazil (Teixeira *et al.,* 2008a; Teixeira *et al.,* 2008b) and efforts have been made to extensively survey Brazilian bees for IAPV. In South America IAPV was first reported in Brazil (Teixeira *et al.*, 2008a) and later in neighbouring Argentina (Reynaldi *et al.*, 2011), showing that this virus is probably present in much of the continent. In this study, we investigated the range of IAPV in Brazilian honey bees, and tested whether this virus is related to honey bee population declines.

We surveyed two hundred colonies of Africanized honey bees from ten apiaries located in Altinópolis (São Paulo State), southeast Brazil. Samples were collected in April (autumn in Brazil), coincident with population declines in hives in that area. Decreased worker bee population could be observed in that area between March and July, with few crawling or dead adult bees around the colony, besides signs of some abnormalities in the brood appearance (ambiguous symptoms like brown to yellowish brood, with aqueous or retracted abdomen). Nevertheless, the defining trait observed by beekeepers is simply the occurrence of significant and unexpected production losses without collapse. Simultaneously, twenty colonies from each apiary were checked for the presence or absence of clinical signs and for dead bees near or around the entrances. Such parameters were used to select, in each apiary, ten apparently "healthy colonies" and ten apparently "sick colonies" to study. Adult bees that were covering the brood area were collected from each colony. These bees were called "healthy inside bees" and "sick inside bees", respectively. Additionally, two other samples of 60 apparently healthy bees from the entrance of the hives ("healthy outside bees") and 35 apparently crawling bees adjacent to colonies ("sick outside bees"), were collected. Ten abdomens

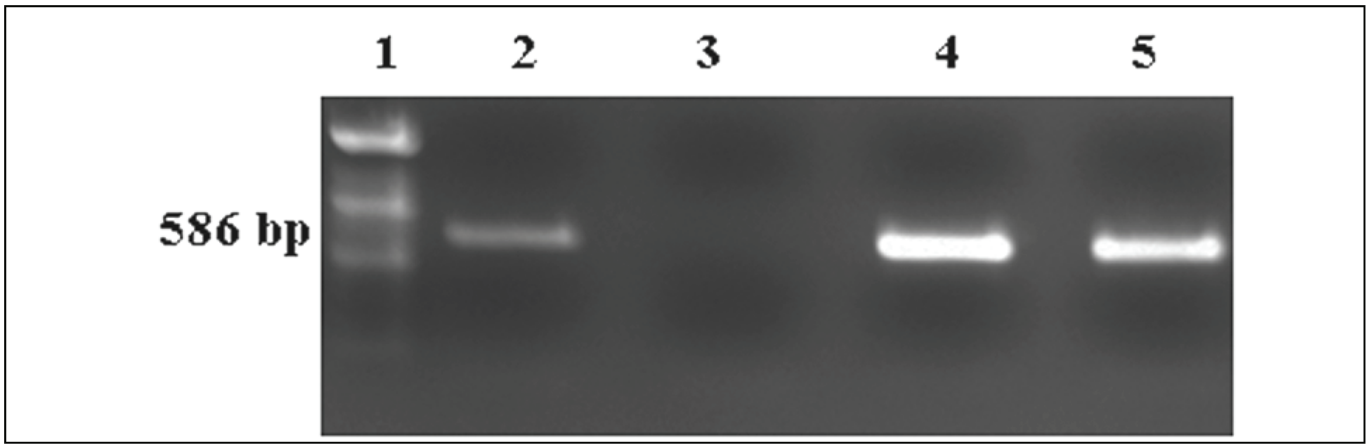

Fig. 1. Electrophoresis of PCR products for bees with IAPV infection. Lane 1: 100 bp DNA ladder (Invitrogen). Lanes 2- Positive control (positive sample from Israel); Lane 3- Negative control; Lane 4 and 5- Positive amplification for IAPV.

from each category of sample were combined in centrifuge tubes, and ground in approximately 7 ml of RNAlater® Tissue Collection buffer (Ambion), prior to shipment to the USA, to limit RNA degradation. After three days at room temperature during shipment, samples were stored at -80^{0}C. Total RNA isolation was performed from a total of 192 colony samples using the RNAqueous®- 96 kit (Ambion) and an initial isolate of 25 µl of the above suspension. Samples were randomly arrayed across two 96-well plates. Extracted RNA was eluted in 50 µl of RNase-free water and then quantified using a spectrophotometer (Pharmacia Biotech, GeneQuant). The cDNA synthesis and PCR conditions was carried out following published protocols (Chen and Evans, 2007). Positive (IAPV positive samples from Israel) and negative controls (water) were included. PCR products were visualized on a 1.7% (w/v) agarose gel containing ethidium bromide (Fig. 1). The specificity of the RT-PCR assay was confirmed by sequencing analysis. The sequence data were analysed using the BLAST server at the National Center for Biotechnology Information, NIH.

Our results showed that 25.7% of all samples from within the colony carried IAPV, although the level of infection between apiaries was variable (from 13.33% to 38.89%) but not significant ($P > 0.05$). IAPV presence was slightly higher in brood area bees from apparently "healthy colonies" (54.35%) than in apparently "sick colonies" (45.65 %), but the observed difference was not significant, nor were there signs of correlation between virus incidence and disease across apiaries. IAPV presence in "sick outside bees" was slightly more frequent than in "healthy outside bees", but this observation was not significant.

Although the mite *Varroa destructor,* linked with colony losses worldwide, and associated with virus transmission, was not a specific focus of the current study, we did find mites in each of the analysed colonies. An evaluation of colonies from this region in 2006 found infestation rates of 10.68 mites per 100 adult bees, on average, and that 9% of worker brood cells contained female mites (152 / 1700, unpublished data).

The introduction of honey bee pathogens into Brazil is a complex subject because of the size and diversity of the country, and its huge border, with contact with many other countries. Most of this border is a free gateway for insects, with no weather and / or physical barrier that could impede migration of honey bees. The best example of that is the case of African honey bees that arrived in Brazil in 1956, and crossed with the European subspecies, spreading quickly across the whole country by the late 1970s. *V. destructor* could also be a source for introduction of IAPV. Clandestine biological material circulation in some parts of Brazil (bee products and queens from other countries) is also common, making it hard to decide whether or not IAPV is endemic in Brazil. More studies with historical samples should be conducted to investigate this question.

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